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THE STRUCTURE OF ZAPOTIN

D. L. DREYER

Fruit and Vegetable Chemistry Laboratory,[1]
263 South Chester Avenue, Pasadena, California 91106

and

D. J. BERTELLI

Department of Chemistry,
University of California, Santa Barbara, Santa Barbara, California

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Abstract—Spectroscopic evidence is presented which indicates that zapotin, a flavone occurring in seeds of *Casimiroa edulis* Llave *et* Lex, is 2',5,6,6'-tetramethoxyflavone. Zapotin thus has a unique 2',6'-dimethoxy B-ring which has not previously been reported among naturally occurring flavonoids.

KINCL *et al.*[2] originally reported the isolation of zapotin, zapotinin and 5,6-dimethoxyflavone[3] from *Casimiroa edulis* Llave *et* Lex (Rutaceae). It was suggested[2] that zapotin and zapotinin were a tetramethoxyflavone or isoflavone and the corresponding 5-dimethyl derivative respectively. Subsequently, the isolation of 2',5,6-trimethoxyflavone was reported[4] from the same plant and zapotin formulated as 2',5,6,7-tetramethoxyflavone.[4] An isoflavone structure for zapotin was rejected on the basis of its stability towards base hydrolysis and lack of identity with the known 2',5,6,7-tetramethoxyisoflavone,[5] although no direct comparison between samples appears to have been reported in spite of the similarity of physical constants. The UV spectrum of zapotin did not show well defined bands characteristic of most flavones, but resembled in some respects that of an isoflavone.[6] The stability of zapotin to strong base has been confirmed in the present study.

There have recently been several syntheses of 2',5,6,7-tetramethoxyflavone[7] and this material proved to have quite different physical properties from those reported for zapotin.[2]

In the present study, a 60 Mc NMR spectrum was helpful in establishing the gross flavone features of zapotin but it was not sufficient to determine the complete structure. Thus, while resonances for H-3 and four MeO groups could be distinguished, the remaining aromatic protons formed a pattern which was not

[1] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

[2] F. A. Kincl, J. Romo, G. Rosenkranz and F. Sondheimer, *J. Chem Soc* 4163 (1956).

[3] J. Iriarte, F. A. Kincl, G. Rosenkranz and F. Sondheimer, *J. Chem Soc.* 4170 (1956).

[4] F. Sondheimer and A. Meisels, *Tetrahedron* **9**, 139 (1960).

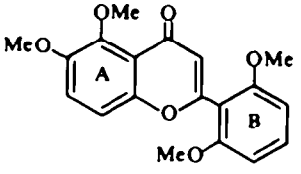
[5] P. Crabbé, P. R. Leeming and C. Djerassi, *J. Am. Chem. Soc.* **80**, 5258 (1958).

[6] L. Jurd, *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman) p. 107. MacMillan, New York (1962).

[7] L. Farkas and M. Nogradi, *Chem. Ber.* **98**, 164 (1965); B. R. Pai, P. S. Subramaniam and V. Subramanyam, *Tetrahedron* **21**, 3573 (1965); V. V. S. Murti, P. V. Raman and T. R. Seshadri, *Curr. Sci.* **34**, 398 (1965); *Chem. Abstr.* **63**, 6956 (1965).

immediately interpretable. The NMR spectrum did not show any usual downfield signal assignable to a 2'- or 6'-proton. Moreover, the singlet with good ringing assignable to H-3 occurred at δ 6·28, about δ 0·3–0·5 upfield from the usual position for such resonances.[8] Resonances were also lacking for a 5-proton confirming the presence of a 5-MeO group. Substantial chemical evidence was previously available on this point.[2,4]

The 100 Mc NMR (Fig. 1) in deuteriochloroform and trifluoroacetic acid was, however, interpretable. The NMR spectra in these solvents leads to the formulation of zapotin as 2',5,6,6'-tetramethoxyflavone (I). The spectrum in deuteriochloroform



I

exhibits an AB pattern from the A-ring protons superimposed on the A part of an AX_2 pattern from the B-ring protons. The AX_2 pattern consists of a one proton

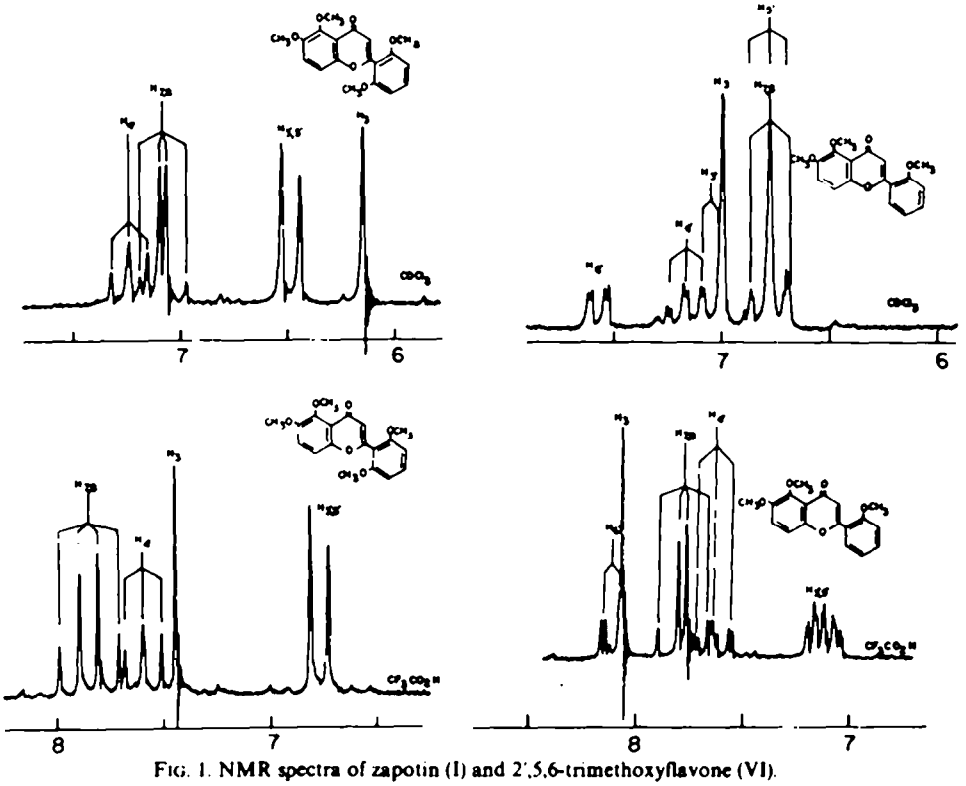


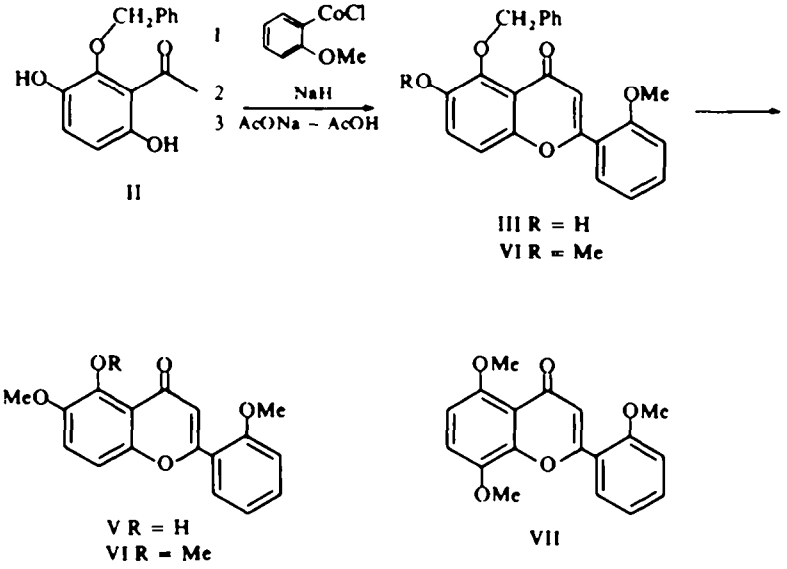
FIG. 1. NMR spectra of zapotin (I) and 2',5,6-trimethoxyflavone (VI).

[8] See, for example, R. M. Horowitz and B. Gentili, *Chem. & Ind.* 498 (1964); R. M. Dawson, C. A. Henrick, P. R. Jefferies and E. J. Middleton, *Aust. J. Chem.* **18**, 1871 (1965); J. Massicot and J. Marthe, *Bull. Soc. Chim. Fr.* 1962 (1962), J. Massicot, J. Marthe and S. Heitz, *Ibid.* 2712 (1963).

downfield triplet and a two proton upfield doublet. Thus, irradiation of the doublet at δ 6·62 collapsed the triplet centered at δ 7·39 into a singlet, but left the AB doublet unchanged. This clearly establishes that there are three hydrogens on the B-ring and two hydrogens on the A-ring. The NMR spectrum of zapotin in trifluoroacetic acid was particularly helpful, wherein the AB doublet of the A-ring protons were shifted far enough downfield to be completely clear of the AX_2 pattern of the B-ring (Fig. 1). This technique may have some general utility in structure determination of flavonoids. In trifluoroacetic acid the carbonyl group is protonated with some positive charge distributed in the A-ring causing the downfield shift of the A-ring protons.

The NMR data presented for zapotin do not uniquely distinguish between 2',5,6,6'- and 2',5,6',8-tetramethoxyflavone structures. The similar chemical shifts of the A-ring resonances for zapotin with those of 2',5,6-trimethoxyflavone (VI)[9] constitutes excellent evidence for the former structure. Furthermore, the difference in chemical shifts between the 6- and 7-resonances of 2'5,8-trimethoxyflavone[9] is δ 0·57 (in $CDCl_3$) in contrast to the much smaller differences in chemical shifts for the 7- and 8-resonances of both zapotin (δ 0·10) and VI (δ 0·04).[10]

In a study of NMR solvent shifts on permethylated and acetylated flavonoids[11] it has been found that methoxy resonances generally occur δ 0·3–0·6 ppm upfield in benzene relative to chloroform. Two exceptions to this rule have been found. Resonances due to methoxy groups in the 6- and 3-positions generally occur in the same place in both chloroform and benzene. Zapotin shows three methoxy resonances occurring well upfield in benzene and one unaffected by the change in solvent from chloroform to benzene. The close model, 2',5,6-trimethoxyflavone (VI), shows only



[9] M. L. Doporto, K. M. Gallagher, J. E. Gowen, A. C. Hughes, E. M. Philbin, T. Swain and T. S. Wheeler, *J. Chem. Soc.* 4249 (1955).

[10] The 6- and 7-resonances in 5,8-dimethoxy-2-methylbenzochromone are also well separated and occurred at δ 6·72 and 7·10 respectively, well upfield from the A-ring resonances in zapotin.

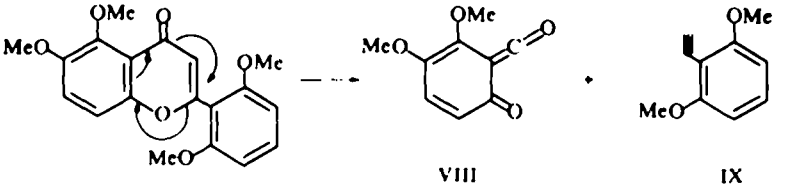
[11] R. M. Horowitz and D. L. Dreyer, unpublished results.

two methoxy resonances occurring upfield in benzene compared to chloroform. Again the position of one methoxy resonance remains unchanged with change in solvent.

The unusual UV spectrum of zapotin can now be ascribed to the nonplanarity of the unique 2',6'-dimethoxysubstituted B-ring with the hetero ring.[12] This also accounts for the upfield position of H-3, which now lies in the positive shielding region of the B-ring. A similar upfield shift of H-2 in a 2',6'-disubstituted isoflavone has been noted by Falshaw *et al.*[13] The 2',6'-disubstituted B-ring, by causing the 2-position to be highly hindered also accounts for the remarkable stability of zapotin to refluxing 20% potassium hydroxide.

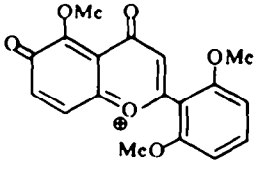
2',5,6-Trimethoxyflavone was synthesized by a standard route (II→VI) using the Baker-Venkataraman rearrangement. Attempted synthesis of zapotin (I) by the same route starting with 2,6-dimethoxybenzoyl chloride and II has, thus far, proven unpromising.

The mass spectra of flavonoids have received relatively little attention. Barnes and Occolowitz[14] showed that fragmentation of the heterocycle ring in a reverse Diels-Alder fashion was important in flavone itself. Further work[15] indicated this path to be of much less importance in substituted cases. Thus, the mass spectrum of zapotin shows only low intensity peaks at *m/e* 180(3) and 162(3) (I → VIII + IX) and lacks peaks at *m/e* 210 and 132 which would be expected for a tetramethoxyflavone with only one methoxy group in the B-ring.



I VIII IX

Although zapotin shows an abundant molecular ion, the base peak is at M-15, indicating the facile loss of a Me group. Mass spectra studies[16] on methoxy coumarins has shown that 5- and 7-MeO derivatives do not easily lose Me groups while 6-methoxycoumarins easily lose Me.[17] The structure of the M-15 peak is best represented as X, again consistent with a 6-MeO group. A peak at *m/e* 163·5(7) appears to be a doubly charged ion of X.



X

[12] C. T. Davis and T. A. Geissman, *J. Amer. Chem. Soc.* **76**, 3507 (1954).
[13] C. P. Falshaw, W. D. Ollis, J. A. Moore and K. Magnus, *Tetrahedron Supplement* **7**, 333 (1966).
[14] C. S. Barnes and J. L. Occolowitz, *Aust. J. Chem.* **17**, 975 (1964).
[15] R. I. Reed and J. M. Wilson, *J. Chem. Soc.* 5949 (1963); A. Pelter, P. Stainton and M. Barber, *J. Heterocyclic Chem.* **2**, 262 (1965).
[16] J. P. Kutney, G. Eigendorf, D. L. Dreyer and I. A. Mitschier, *Canad. J. Chem.* in press.
[17] R. H. Shapiro and C. Djerassi, *J. Org. Chem.* **30**, 955 (1965).

The evidence thus suggests that zapotin is a unique 2',6'-dimethoxyflavone and is closely related biogenetically to the other flavones occurring in *C. edulis*.[2,4]

On the basis of extensive chemical studies, King *et al.*[18] have formulated oxyayanin A as 2',5,5'-trihydroxy-3,4',7-trimethoxyflavone. The question of the structure of oxyayanin A has recently been reopened by Jain *et al.*[19] in a recent synthesis of this material, which indicates that it is not identical with oxyayanin A. Two unusual properties of oxyayanin A are similar to those of zapotin; (a) it shows remarkable stability towards refluxing 20% KOH and (b) the long wavelength UV band exhibits very low intensity (λ_{max}^{EtOH} 258 (4·06), 302 (3·74), 349 (3·49) mμ (log ε)).[18] These observations are difficult to reconcile with the other results of King *et al.*[18] but do suggest that a 2',6'-disubstituted B-ring might be considered for oxyayanin A.

EXPERIMENTAL

Isolation of zapotin (I). Dried and ground zapote seeds were extracted with acetone. Solvent was removed from the extracts and the residue was chromatographed on acid washed alumina. The content of the eluted fractions was monitored by TLC, using silicic acid absorbant and a 1:1 $CHCl_3$ AcOEt solvent system. Zapotin was detected by exposing the plates to HCl gas with which zapotin gives a bright yellow spot. The fractions containing zapotin were pooled, the solvent was removed, and the residue crystallized from MeOH to give zapotin, m.p. 147–148°: ν 1654, 1588 cm^{-1} (Nujol); λ_{max}^{EtOH} 228 (29,000), 257 (17,000), 325 (7,400) mμ; NMR δ 7·39(t) J = 8 H-4'; 7·29; 7·19 (AB doublet) J = 8 H-7 and H-8; 6·62 (d) J = 8 H-3' and H-5'; 6·28 (s) H-3; 3·97; 3·90; 3·77; 3·77 methoxys ppm ($CDCl_3$); δ 3·93; 3·50; 3·40; 3·40; ppm (benzene); δ 7·93; 7·77 (AB doublet) J = 9 H-7 and H-8; 7·60 (t) J = 8 H-4'; 7·43 (s) H-3; 6·77 (d) J = 8 H-3' and H-5'; 4·45; 4·11; 3·97; 3·94 MeO ppm (trifluoroacetic acid). Mass spect. peaks occurred at *m/e* 41(10), 42(3), 43(14), 44(14), 45(7), 50(3), 51(3), 53(5), 54(3), 55(8), 56(3), 57(8), 59(3), 60(5), 61(3), 62(3), 63(5), 65(5), 66(3), 67(5), 68(3), 69(7), 70(3), 71(5), 73(3), 74(3), 76(5), 77(5), 78(5), 79(5), 81(5), 82(3), 83(5), 91(5), 94(3), 95(3), 97(3), 105(3), 107(3), 109(7), 119(3), 121(3), 131(3), 135(8), 136(3), 137(20), 147(3), 148(7), 149(5), 150(3), 151(3), 161(3), 162(3), 163·5(7), 166(16), 180(3), 253(5), 266(3), 267(3), 269(3), 282(3), 283(3), 284(3), 295(3), 297(10), 298(3), 299(3), 309(3), 311(8), 312(11), 313(11), 314(3), 325(3), 327(100), 328(21), 329(3), 341(3), 342(57), 343(13). (Found: C, 66·5; H, 5·2. $C_{19}H_{18}O_6$ requires: C, 66·65; H, 5·3%.)

2'-Methoxy-5-benzyloxy-6-hydroxyflavone (III). A mixture of 0·5 g of II[21] and 2 g O-methoxybenzoyl chloride in pyridine was allowed to stand overnight. The mixture was diluted with water and extracted with benzene. The benzene extracts were washed with 5% Na_2CO_3 aq and 20% HCl aq. The dried benzene extracts were filtered through a short column of alumina. Removal of solvent from the filtrates gave an oil which could not be induced to crystallize and so was used directly in the next step. The diester was dissolved in dioxan and excess 50% NaH in oil added. After standing overnight in a stoppered flask the mixture was decomposed with ice water and the soln extracted with ether. The aqueous phase was then acidified and the product collected by extraction with ether. Solvent was removed from the ether extracts and the residue refluxed with AcOH AcONa for 6 hr. The soln was diluted, extracted with ether and the ether extracts were washed with 5% Na_2CO_3 aq. Removal of solvent after drying gave a residue which was recrystallized from benzene; m.p. 171–174°. λ_{max}^{EtOH} ~232 (12,800), 272 (16,000), 325 (10,000) mμ; NMR δ 7·85 (q) J = 8, J = 2 H-6'; 7·32 (s) benzyl aromatics; 7·07 (s) H-3; 5·20 (s) benzyl methylene; 3·87 MeO ppm ($CDCl_3$). (Found: C, 73·4; H, 4·92. $C_{23}H_{18}O_5$ requires: C, 73·78; H, 4·85%.)

2',6-Dimethoxy-5-benzyloxyflavone (IV). To a soln of 200 mg of III in 40 ml THF was added excess diazomethane in ether. After 0·5 hr excess diazomethane and solvent were removed under an air jet and the residue filtered through a short column of alumina with benzene. Removal of solvent from the filtrates gave a residue, m.p. 94·5–96°, after crystallization three times from MeOH. λ_{max}^{EtOH} 235 (18,500), 268 (23,000), 326 (16,000); NMR δ 7·85 (q) J = 8, J = 2 H-6'; 7·27 (s) benzyl aromatics; 7·00 (s) H-3; 5·15 (s) benzyl

[18] F. E. King, T. J. King and P. J. Stokes, *J. Chem. Soc.* 4587 (1954).

[19] A. C. Jain, S. K. Mathur and T. R. Seshadri, *Indian J. Chem.* 4, 364 (1966).

[20] NMR data were taken at 60 and 100 Mc/s and are given in δ relative to internal tetramethylsilane. The relative areas of peaks were consistent with the assignments.

[21] W. Baker, N. C. Brown and J. A. Scott, *J. Chem. Soc.* 1922 (1939).

methylene; 3·90; 3·85 MeO's ppm ($CDCl_3$). (Found: C, 74·1; H, 5·24. $C_{24}H_{20}O_5$ requires: C, 74·21; H, 5·19%.)

5-Hydroxy-2',6-dimethoxyflavone (V). A soln of 30 mg of IV in 2 ml glacial AcOH and 1 ml conc HCl was heated on a steam bath for 30 min. The soln was then cooled, water added to the cloud point and the resulting soln cooled further in an ice bath; product collected and recrystallized from MeOH, m.p. 153–154°; green $FeCl_3$. λ_{max}^{EtOH} ~234 (14,400), 277 (27,000), 330 (13,800) mμ; NMR δ 7·85 (q) J = 8, J = 2 H-6'; 7·23 (d) J = 8 H-8; 6·90 (d) J = 8 H-7; 7·02 (s) H-3; 3·95; 3·95 MeO's ppm ($CDCl_3$). (Found: C, 68·2; H, 4·73. $C_{17}H_{14}O_5$ requires: C, 68·45; H, 4·73%.)

2',5,6-Trimethoxyflavone (VI). Compound V was methylated with excess Me_2SO_4 and 20% NaOH aq. The product was recovered by extraction with ether and recrystallized from AcOEt–hexane, m.p. 124–125°; lit.[9] m.p. 124–125°; λ_{max}^{EtOH} 235, 267, 326 mμ NMR[22] δ 7·58 (q) J = 8, J = 2 H-6'; 7·18 (m) H-4'; 7·05 (m) H-3'; 7·00 (s) H-3; 6·80; 6·76 (AB doublet) J = 9 H-7 and H-8; 6·78 (m) H-5'; 3·98; 3·90; 3·90 MeO's ppm ($CDCl_3$); δ 4·03; 3·57; 3·27 MeSO's ppm (benzene).

2',5,8-Trimethoxyflavone (VII). This material was prepared from 2,5-dimethoxy-6-hydroxyacetophenone[21] and O-methoxybenzoyl chloride in the same manner as III; m.p. 200–201°; lit.[9] m.p. 200–201°; λ_{max}^{EtOH} ~224 (17,000), 271 (18,300), 324 (14,100) mμ; NMR δ 8·02 (q) J = 7, J = 2 H-6'; 7·23 (d) J = 9 H-7; 7·13 (s) H-3; 6·66 (d) J = 9 H-6; 3·93; 3·93; 3·93 MeO's ($CDCl_3$); δ 3·57; 3·40; 3·12 MeO's (benzene).

5,8-Dimethoxy-2-methylbenzochromone NMR δ 7·10 (d) J = 9 H-7; 6·72 (d) J = 9 H-6; 6·08 (s) H-3; 3·90; 3·90 MeO's, 2·33 Me ppm ($CDCl_3$).

Acknowledgements- The authors are indebted to L. M. White for the analytical data and to Dr. R. M. Horowitz for helpful discussions.

Note added in proof. P. J. Garratt, F. Scheinmann and F. Sondheimer [*Tetrahedron* **23**, 2413 (1967)] have recently shown the identical structure for zapotin by reasoning similar to that employed here.

[22] These assignments were confirmed, where possible, by spin decoupling.